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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	
Yong-Li Ruan et al.	Group Art Unit: 1638
Application No.: 10/003,405	Examiner: RUSSELL KALLIS
Filed: December 6, 2001	Confirmation No.: 5391
For: MODIFICATION OF SUCROSE SYNTHASE GENE EXPRESSION IN PLANT TISSUE AND USES THEREFOR	

Declaration of Tony Arioli

I, Tony Arioli, hereby declare that:

1. I am a citizen of Australia, residing currently in Belgium.

2. I received a PhD degree in 1992 from the Australian National University .

3. Since 1999, I have been employed by Bayer CropScience PTY Ltd., Australia, and currently I am temporarily attached to Bayer BioScience N.V., Belgium ("BBS N.V."). My work at BBS N.V. currently involves the supervision of research projects involving fiber improvement in cotton plants

4. I am familiar with the field of the plant molecular biology, particularly the fields of cellulose and fiber production in plants such as cotton. I have authored and co-authored several scientific publications in this field (see APPENDIX 1).

5. I have read US patent application 10/003,405 ("the Application"). I have been informed Bayer has a license to the Application.

6. I have also been informed that in the Official Action, dated June 30, 2004, the Examiner has rejected claims 1-3, 8-17 and 21-30 of the Application.

7. It is my understanding that the Examiner believes that these claims contain

"subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains to make and/or use the invention". In particular, I understand that the Examiner maintains that

- the specification provides guidance only for reducing fiber and seed development in cotton transformed with antisense and co-suppression constructs comprising an undefined polynucleotide encoding an unspecified sucrose synthase;

- the specification fails to provide guidance for using the other polynucleotides encompassed by the claims that encode an active sucrose synthase that would alter fiber development, improve fiber yield, improve fiber quality or increase seed size by providing cells of plants with a polynucleotide capable of being translated into an active sucrose synthase;

- the specification fails to teach which sucrose synthase encoding polynucleotides would alter fiber development other than SEQ ID No. 1;

- the specification does not teach any increases or improvements in fiber quality or size;

- the state of the art for transformation of a plant with a sucrose synthase encoding polynucleotide in order to increase or alter fiber development is unpredictable because there are multiple isoforms of sucrose synthase that are drawn to somewhat different enzymatic activities, producing different products in different plant cells in different plant organs.

I respectfully disagree with the Examiner.

8. Transgenic cotton plant lines were produced, which, according to teaching of the specification, comprise a chimeric gene encoding a sucrose synthase protein. Several of these cotton plant lines exhibit an increased fiber length, correlated with the increased sucrose synthase activity.

9. A T-DNA vector (pTDL014) was constructed using standard recombinant DNA procedures in accordance with Example 3 of the present application. This T-DNA comprises a potato sucrose synthase coding region under control of the S7 promoter of the subclover stunt virus and 3' untranslated region of the segment 5 dsRNA circle of the Subclover Stunt Virus. The T-DNA further comprises a selective marker gene. A figure representing pTDL014 can be found in APPENDIX II.

6. The T-DNA vector was introduced in *Agrobacterium tumefaciens* and the resulting *Agrobacterium* strain was used for transformation of cotton, essentially as described at page 25 of the application. Three lines of T0 sucrose synthase overexpression lines were selected for comprehensive molecular and biochemical analysis in the T1 generation. The lines exhibited various degrees of increase in sucrose synthase expression and apart from line 2 are single-copy plants. Comparative analysis was performed on a segregating population comprising segregants which do not contain the transgene but are otherwise isogenic and have the same tissue culture history as the transgenic plants.

7. Transgenic cotton plants obtained by selfing of the T0 generation of the mentioned three lines were germinated and further grown, These T1 generation plants were subjected to the following analyses:
    a. the presence of the sucrose synthase overexpressing transgene was scored by PCR amplification;
    b. sucrose synthase activity in fibers from seed 20 days after anthesis ("DAA") was determined;
    c. relative abundance of potato sucrose synthase mRNA in fiber cells from 20 DAA seed was determined;

d. acid and alkaline invertase activity in fiber cells from 20 DAA seed was determined;
e. fiber length was determined according to Schubert et al. (1973) *Crop Science* 13: 704-709 from 20 DAA seed.

The results of these tests for three different lines are summarized in APPENDIX III. Briefly, sucrose synthase activity in fiber cells can be increased in transgenic cotton plants comprising a sucrose synthase gene according to the claimed invention, and such increased sucrose synthase activity correlates positively with increased fiber length at 20 DAA when compared to the non-transgenic segregating plants.

8. It is my opinion that these results rebut the Examiner's arguments noted above. More particularly, the guidance provided by the application is not limited to reduction of fiber and seed development in cotton transformed with antisense and co-suppression constructs directed towards the endogenous sucrose synthase gene. As indicated at least on page 29 of the application (in conjunction with Figure 8C), the linear correlation between fiber length and sucrose synthase activity up to the wild type level provided support for the concept that overexpression of sucrose synthase activity above wild type level would lead to fiber lengths above the fiber length of control plants. Example 3 (page 30) provides explicit guidance for the construction of a chimeric gene leading to increased sucrose synthase activity in fiber cells, using a potato sucrose synthase coding region under control of a subterranean clover stunt virus promoter (S7 promoter). The experimental results provided herein confirm the teaching of Example 3. Therefore, it is my opinion that
   - the application provides guidance for using other polynucleotides encompassed by the claims that encode an active sucrose synthase to alter fiber development and improve fiber quality by providing cells of plants with a polynucleotide capable of being translated into an active sucrose synthase;
   - the application teaches that sucrose synthase encoding

polynucleotides other than SEQ ID No. 1 alter fiber development;

- the application teaches an increase in fiber size;

9. The Examiner argues that the existence of multiple isoforms of sucrose synthase, with slightly different enzymatic activities and expression pattern, contributes to the unpredictability of the transformation of plants with a sucrose synthase encoding polynucleotide in order to increase or alter fiber development. I agree with the Examiner that multiple isoforms of sucrose synthase exist in plants, and that some may be involved in providing carbon for cellulose biosynthesis, while others may be involved in providing carbon for starch biosynthesis. However, it appears from experimental results according to Example 3 of the patent application that such differences are not critical to achieve fiber length increase. Indeed, the over-expression in cotton fiber cells of a sucrose synthase isoform normally expressed in tubers from potatoes and normally involved in starch biosynthesis achieves the goals of the invention. Therefore, it is my opinion that a pivotal feature of the invention for the increase of fiber length is the increase in the level of sucrose synthase in fiber cells. In my opinion, the normal fate and expression pattern of the enzymatic isoform of sucrose synthase actually used to increase the fiber length is not critical .

10. From my review of the application, and my understanding of the state of the art, It is also my opinion that it would not require more than routine experimentation for the ordinary scientist working in this area to make and use the invention of the rejected claims.

I also declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false

statements may jeopardise the validity of this application or any patent issued thereon.

23 12 2004 _____

Date

Tony Arioli

APPENDIX I

1. **Burn JE, Hurley UA, Birch RJ, Arioli T, Cork A, Williamson RE.**
The cellulose-deficient Arabidopsis mutant rsw3 is defective in a gene encoding a putative glucosidase II, an enzyme processing N-glycans during ER quality control.
Plant J. 2002; **32**(6):949-60.

2. **Williamson RE, Burn JE, Birch R, Baskin TI, Arioli T, Betzner AS, Cork A.**
Morphology of rsw1, a cellulose-deficient mutant of Arabidopsis thaliana.
Protoplasma. 2001; **215**(1-4):116-27.

3. **Lane DR, Wiedemeier A, Peng L, Hofte H, Vernhettes S, Desprez T, Hocart CH, Birch RJ, Baskin TI, Burn JE, Arioli T, Betzner AS, Williamson RE.**
Temperature-sensitive alleles of RSW2 link the KORRIGAN endo-1,4-beta-glucanase to cellulose synthesis and cytokinesis in Arabidopsis.
Plant Physiol. 2001; **126**(1):278-88.

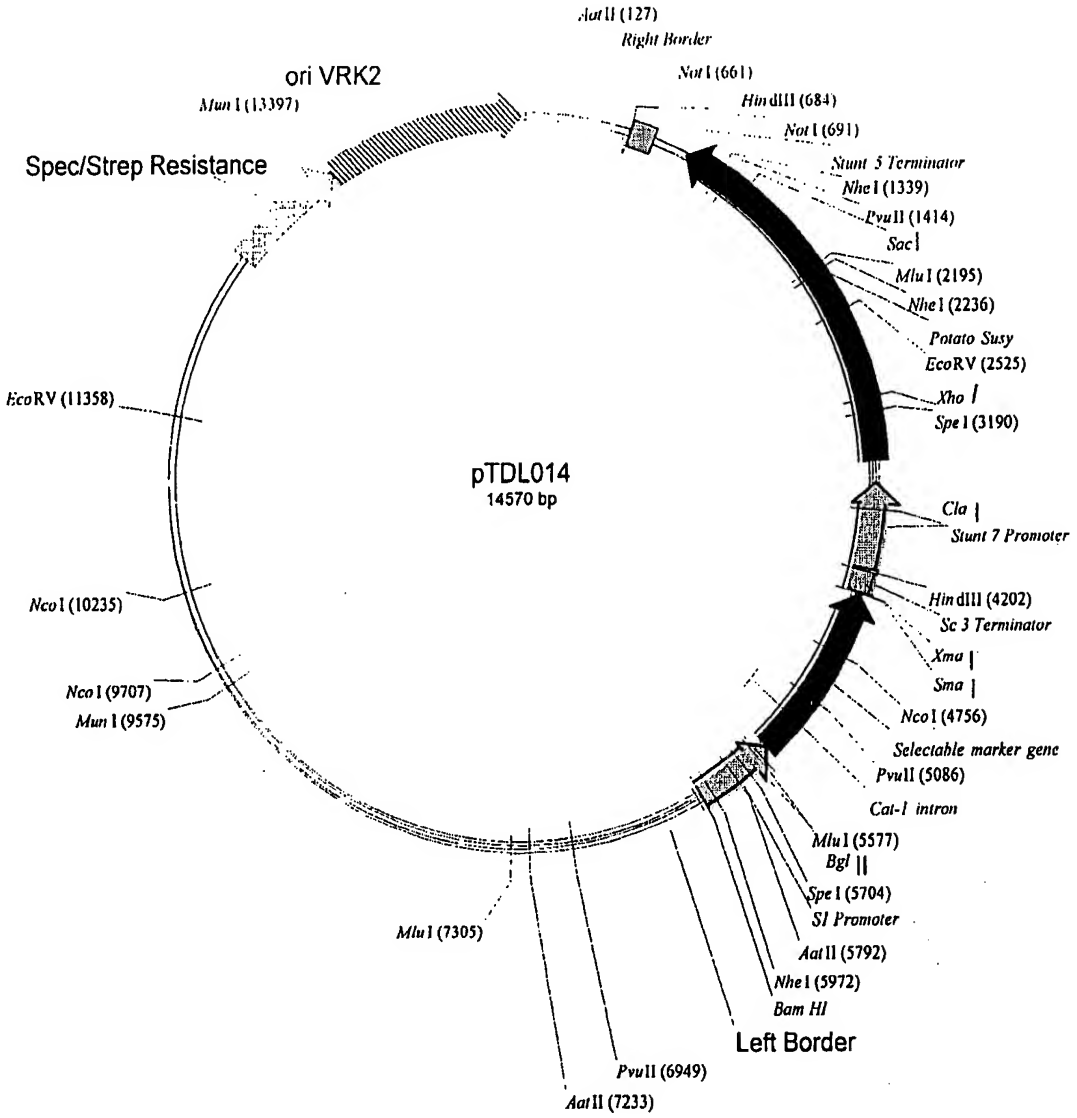
4. **Arioli T, Peng L, Betzner AS, Burn J, Wittke W, Herth W, Camilleri C, Hofte H, Plazinski J, Birch R, Cork A, Glover J, Redmond J, Williamson RE.**
Molecular analysis of cellulose biosynthesis in Arabidopsis.
Science. 1998; **279**(5351):717-20.

5. **Howles PA, Arioli T, Weinman JJ**
Nucleotide sequence of additional members of the gene family encoding chalcone synthase in Trifolium subterraneum.
Plant Physiol. 1995; **107**(3):1035-6

6. **Howles PA, Arioli T, Weinman JJ**
Characterization of a phenylalanine ammonia-lyase multigene family in Trifolium subterraneum.
Gene. 1994; **138**(1-2):87-92.

7. **Arioli T, Howles PA, Weinman JJ, Rolfe BG**

In Trifolium subterraneum, chalcone synthase is encoded by a multigene family. Gene. 1994; **138**(1-2):79-86.


AatII (127)
Right Border
NotI (661)
HindIII (684)
NotI (691)
Stunt 5 Terminator
NheI (1339)
PvuII (1414)
SacI
MluI (2195)
NheI (2236)
Potato Susy
EcoRV (2525)
XhoI
SpeI (3190)
ClaI
Stunt 7 Promoter
HindIII (4202)
Sc 3 Terminator
XmaI
SmaI
NcoI (4756)
Selectable marker gene
PvuII (5086)
Cat-1 intron
MluI (5577)
BglII
SpeI (5704)
SI Promoter
AatII (5792)
NheI (5972)
Bam HI
Left Border
PvuII (6949)
AatII (7233)
MluI (7305)
NcoI (9707)
MunI (9575)
NcoI (10235)
EcoRV (11358)
MunI (13397)
ori VRK2
Spec/Strep Resistance
pTDL014
14570 bp

APPENDIX II

APPENDIX III

Transgenic cotton line	PCR	Transgene copy number	mRNA level	Activity in fiber from 20DAA seed (nmol.mg^{-1}protein.min^{-1})			Fiber length (cm)
				Sucrose synthase	Acid invertase	Alkaline invertase	
Line 1-plant 1	-	0	--	577.5	1131.7	112.3	2.2
Line 1-plant 2	+	1	+	1064.0	1405.2	155.2	2.8
Line 2-plant 1	-	0	--	868.8	1766.4	240.0	3.1
Line 2-plant 2	+	2	++++	1612.2	2427.5	292.4	3.6
Line 3 plant 1	-	0	--	538.0	927.3	194.5	3.1
Line 3 plant 2	+	1	++++	867.8	1392.4	260.4	3.5

mRNA level is visually scored for relative abundance of the potato Susy mRNA relative to the mRNA of the endogenous Sucrose synthase gene.

Each value is the mean of three replicates, with standard error below 12% of the mean. Each value of fiber length is the mean of 5 replicates with standard error below 9% of the mean.